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Uptake of Bromacil by Isolated Barley Roots

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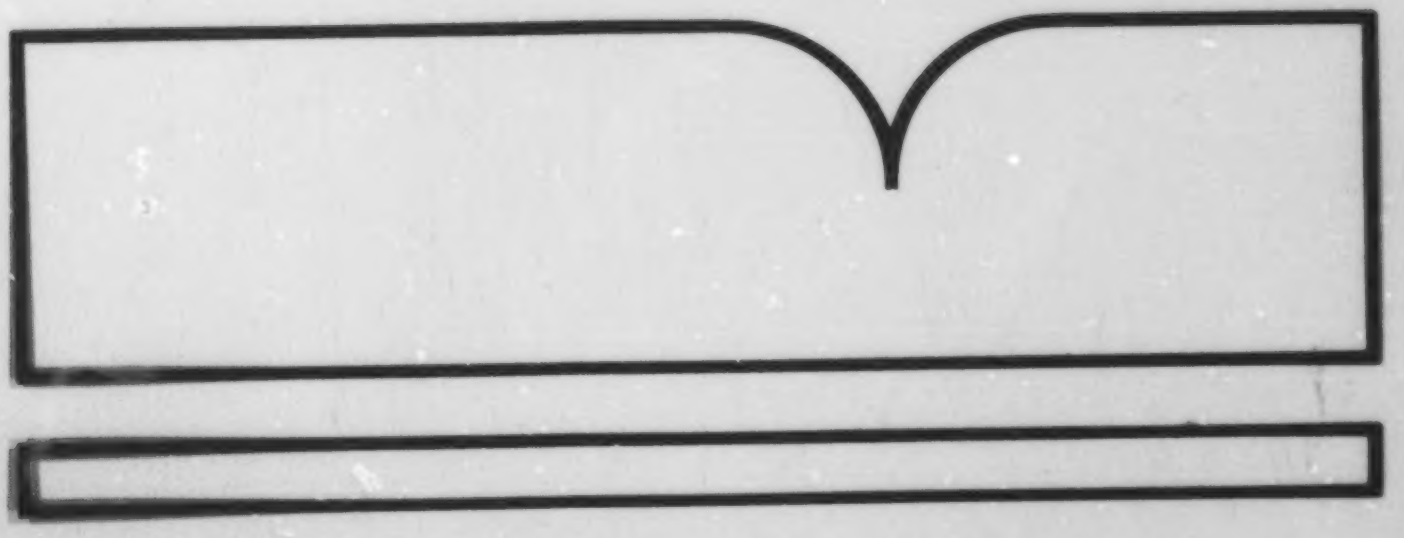

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16. ABSTRACT

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JOURNAL ARTICLE

UPTAKE OF BROMACIL BY ISOLATED BARLEY ROOTS

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Abstract. A study of bromacil uptake by excised barley (*Hordeum vulgare*) roots was used to evaluate this procedure as a tool to learn the uptake characteristics of toxic organic chemicals. Bromacil uptake was shown to be a passive process with an uptake rate (at 0.8 mg l^{-1}) of 0.64 μg bromacil g^{-1} fresh root hr^{-1}. A Q_{10} for the process was determined to be 1.5 and living roots were required for bromacil uptake. This procedure was judged to be a quick and inexpensive method to screen plant uptake of toxic chemicals.

1. Introduction

A technique using isolated barley roots was developed by Hoagland and Broyer (1936) to learn the basic principles of plant mineral nutrition. Variations in treatment and sampling by other investigators have yielded information about: (1) element interactions; (2) oxygen dependence; (3) membrane exclusion; (4) differential uptake; (5) mineral discrimination; and (6) the essential nature of some elements (Epstein and Hagen, 1952; Epstein, 1961; Waisel, 1962). Biphasic uptake patterns were identified for certain cations (Epstein *et al.*, 1963) and multiphasic patterns were identified for some anions (Nissen, 1971; Vange *et al.*, 1974). Some threshold concentrations were identified and uptake rates were determined in the above experiments. This procedure was also used to examine the effects of temperature, pH, metabolic inhibitors, and pesticidal organic compounds (Schmid *et al.*, 1965; Colclasure and Schmid, 1974; Bingham *et al.*, 1970; Maas *et al.*, 1968; Cutler and Rains, 1974; Donaldson *et al.*, 1973). Surprisingly, few organic compounds have been studied; 2,4-D and monuron are notable exceptions (Donaldson *et al.*, 1973). Nevertheless, the extensive data on mineral uptake represents a valuable resource for our work to understand the patterns of uptake of organic compounds by plants.

Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) was selected for initial testing because: (1) it is readily available in radioactively labeled form; (2) it is water soluble; (3) it is relatively stable in aqueous solution; and (4) there is extensive information about its field use and plant uptake characteristics. Bromacil is a general purpose, non-selective herbicide particularly useful in citrus orchards and pineapple fields as well as non-croplands areas for control of a wide range of annual and perennial weeds (Herbicide Handbook Committee, 1983). This herbicide is degraded in the soil by certain bacteria (Rhodes *et al.*, 1970), yet it is relatively persistent and has an approximate half-life of 5–8 months (Gardiner *et al.*, 1969; Leistra *et al.*, 1975). In the field, uptake of bromacil by plants is generally small, dependent upon the rate of transpiration, and varies between species (Gardiner *et al.*, 1969; Shriver and Bingham, 1973; Jordan and Clerx, 1981).

The objectives of this experiment were to: (1) determine the applicability of the

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isolated root procedure to studies of pesticidal and non-pesticidal organic chemical uptake; (2) develop the procedure as a reliable method of categorizing organic compounds which require whole-plant uptake tests; and (3) obtain data on the uptake of bromacil by isolated roots.

2. Methods

Barley (*Hordeum vulgare*, cv. Klages) seeds (4 g) were placed in a 1-l erlenmeyer flask and submerged in aerated deionized water for 24 hr. Pre-germinated seeds were removed from the flask and placed on cheesecloth supported by a stainless steel screen over one-fourth strength nutrient solution (Downs and Helmers, 1975) in a plastic dishpan (30 × 23 × 12 cm). The edges of the cheesecloth were draped over the edges of the screen to provide moisture to the seeds by capillarity. The nutrient solution was aerated with compressed air through numerous holes in a piece of tygon tubing held to the bottom of the dishpan in spiral configuration by interior stainless steel wire. The seeds were covered with a second layer of cheesecloth which was also draped into the nutrient solution. The dishpan was covered with aluminum foil to reduce evaporation and to keep the seeds in the dark. The nutrient was renewed every 3rd day. On the 7th day, a scalpel was used to excise the roots just below the screen to obtain experimental material. Most experiments were conducted at room temperature (22 °C).

Four-gram portions of excised roots were placed in erlenmeyer flasks with 400 ml of nutrient solution containing a pre-determined quantity of test chemical (Figure 1). Each solution had been saturated with pure O_2 by bubbling at the rate of 1 ml min^{-1} with bottled O_2 gas. Each solution was bubbled with O_2-enriched air to stir the roots and maintain oxygen. Pressure in the flask caused by increasing gas volume was relieved through a series of 3 filter tubes containing 13 cc each of ascarite, activated charcoal, and open-cell polyurethane separated by glass wool plugs. Air recirculation was used to minimize loss of any aerosol, volatilized test chemical, or metabolite product. Such materials can leave the system only through filters designed to remove ^{14}C-labeled organic compounds from the air. Experiments were conducted in triplicate.

The ^{14}C-2-bromacil was obtained from New England Nuclear (specific activity 23.8 μCi mg^{-1}).* A stock solution of bromacil in nutrient solution was prepared and stored frozen when not in use.

Temperature treatments were created by placing the flasks in different controlled-temperature water baths. Exposure times varied from 30 min to 24 hr and bromacil concentrations varied from 0.1 to 1.6 mg l^{-1}. After exposure, the solution was poured into a Buchner funnel. Roots were washed twice with 400 ml of 0.2 mM calcium nitrate solution. The washing procedure consisted of swirling the roots with the liquid for 30 sec, then the solution was decanted and sampled.

Roots were removed from the flask, blotted dry for 10 sec, weighed fresh, and

* Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

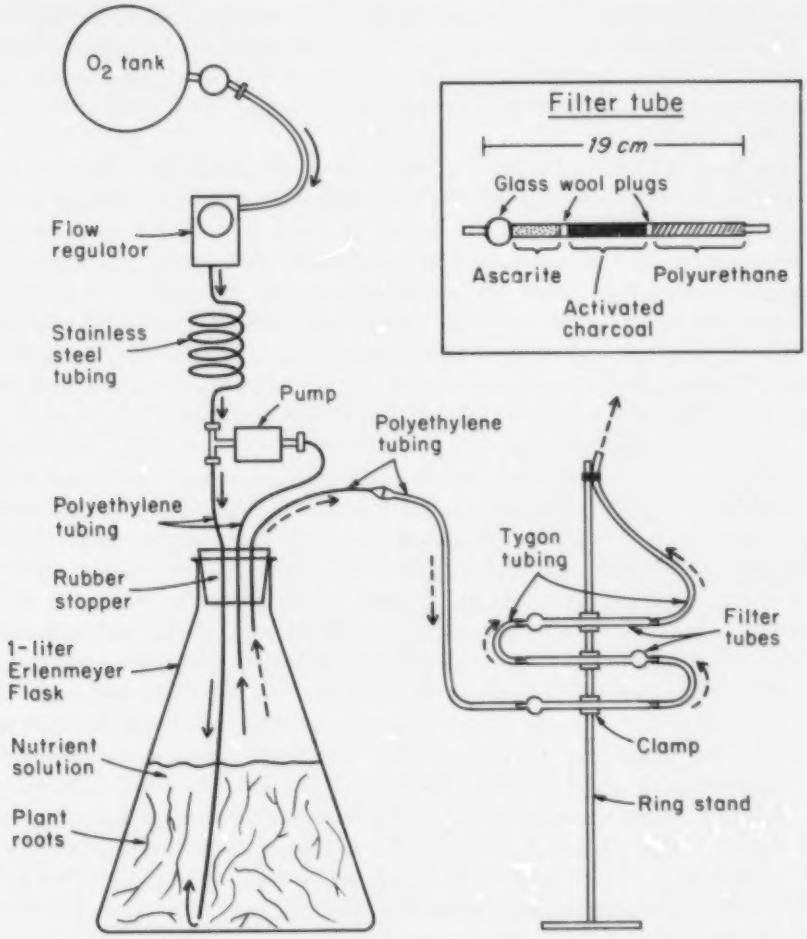

Fig. 1. Exposure apparatus for treating isolated barley roots with bromacil.

oxidized in a combustion oxidizer. Standard samples containing known ^{14}C are ignited in the oxidizer preceding each sampling period to check the overall efficiency of the oxidation and counting of each sample. The roots were oxidized in 0.5 to 0.9 g portions until the total contents of the flask were oxidized. The ^{14}C-labeled carbon dioxide resulting from the oxidation was collected in 9 ml of absorbing solution and 13 ml of scintillation cocktail was added (Collins and Gile, 1981). The carbon dioxide absorbing solution was Oxisorb while Oxiprep-2 was the scintillation cocktail; both were obtained from New England Nuclear* and mixed in proportions recommended by

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the manufacturer for plant and animal samples after combustion and absorption of the $^{14}CO_2$ in a Packard Sample Oxidizer. Oxisorb is an organic base compound used to trap $^{14}CO_2$ in the Packard Sample Oxidizer Model 306*; oxiprep-2 is a pre-mixed scintillation counting solution used for assay of the trapped $^{14}CO_2$ in the same oxidizer model.

The stock solution and exposure solutions were sampled in 8-ml volumes before beginning the experiment; radioactivity was measured in a liquid scintillation counter. Each exposure solution, prepared by diluting the stock, was sampled before and after root exposure. All wash solutions were sampled after the washing procedure. These aqueous samples were mixed with scintillation cocktail and analyzed in a Packard Tri-Carb 460 liquid scintillation counter along with samples collected from the combustion oxidation procedure. The operating parameters are recorded in the instrument log book before counting is initiated. Samples are counted to at least a 1.0% confidence interval (10000 gross counts or more) on the appropriate channel.

To determine actual disintegration rates, the internal standard method was used, in which the samples are counted, spiked with a known amount of activity, and then recounted. Alternatively, the external standard method which utilizes the instrument's automatic external standardization method (AES) was used. The AES ratio method is a specialized channels ratio method in which it is necessary to develop a calibration curve using quenched standards in the same cocktail in which the samples will be counted. A plot of counting efficiency versus AES ratio was made, and the unknown sample efficiencies were determined from their AES ratios. Since the curves are only linear to AES ratios of 0.4, it was necessary to use the internal standard method for samples whose AES ratios fell below that value. New AES ratio curves are made each month. Consistency of instrument operation is determined weekly using sealed quenched standards supplied by New England Nuclear* or Packard Instrument Company.*

Dissolved oxygen concentration was measured with a dissolved oxygen meter while stirring the nutrient solution with a magnetic bar. The concentration was recorded from the meter scale or a strip chart recorder. The flasks were maintained at 22 °C by varying the flow of tap water around them; temperature was monitored with a partially immersed thermometer. The procedure was conducted with and without roots in the exposure flask. The bromacil used for dissolved oxygen measurements was not radioactively labeled.

3. Results and Discussion

The results of a time course study of bromacil uptake by excised barley roots show that there was an initial rapid uptake from the 0.8 $\mu g\, g^{-1}$ solution followed by a period of no further accumulation (Figure 2). The solution concentration was selected from a review of pertinent literature. The maximum concentration of bromacil in the roots was

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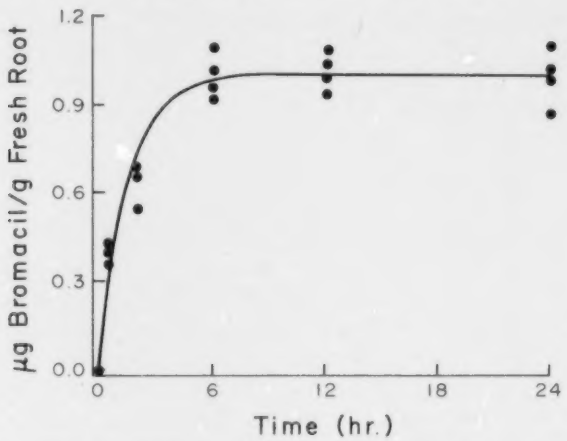

Fig. 2. Regression curve of the uptake of bromacil from a 0.8 mg l^{-1} solution by isolated barley roots with time. The curve is defined by function $y = a(1 - e^{bt})$.

1.01 µg g^{-1} fresh root weight. This concentration is similar to that in the solution and thus indicates a passive uptake.

The uptake rate was determined from the measurements which yield a curve (Figure 2) described by the following regression function

$$y = a(1 - e^{-bt})$$

where a is the maximum value of y (concentration of bromacil in the roots); b is the uptake rate parameter (hr^{-1}); and t is time in hours. The derivative of the above function y with the respect to time (t) gives the rates of bromacil uptake

$$\frac{dy}{dt} = abe^{-bt}.$$

Using this formula, the maximum rate of uptake (at $t = 0$) was determined to be 0.64 µg g^{-1} hr^{-1}.

The amount of test chemical in the exposure solutions decreased less than 4% during treatment of the barley roots (Table I). To understand the uptake kinetics, the treatment conditions must remain constant, a situation provided by the large solution-to-root volume ratio.

Next, a series of tests were conducted to examine the effect of different solution concentrations on the uptake rate. A 2-hr exposure was selected because it was within the rapid uptake period observed in Figure 2 and solution concentrations between 0.1 and 1.6 mg l^{-1} were selected (Figure 3). The relationship between solution concentration and the initial uptake rate approached linearity and as discussed by Donaldson *et al.* (1973) indicates that physical laws such as diffusion and mass flow are controlling uptake and that active uptake was not involved.

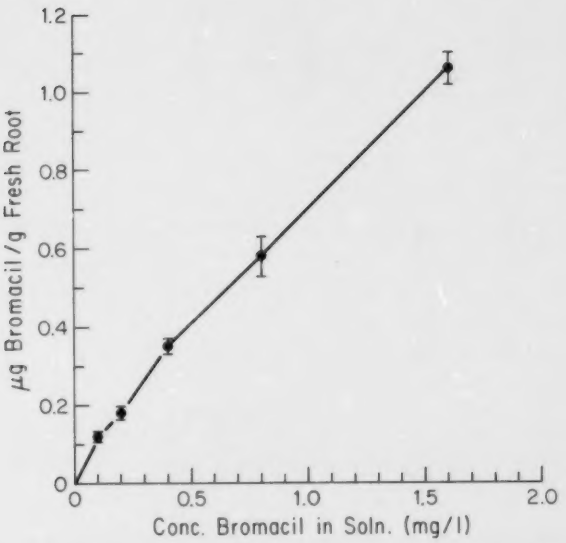

Fig. 3. The relationship between concentration of bromacil in solution and uptake of bromacil by isolated barley roots. A 2-hr exposure was used.

TABLE I

The amount of radioactivity in exposure solutions, before and after treatment of barley roots for varying lengths of time, and in the calcium nitrate wash solutions. The concentration of bromacil in the exposure solution was 0.8 mg l^{-1}

Duration of exposure (hr)	Radioactivity in exposure solution (dpm ml^{-1})		Percent decrease	Percent absorbed radioactivity washed from roots	
	Before exposure	After exposure		First	Second
0.5	20370	19856	2.5	0.8	0.04
2	20370	19897	2.3	0.5	0.05
6	20370	19834	2.6	0.3	0.03
12	20370	19619	3.7	0.3	0.04
24	20370	19879	2.4	0.4	0.03

Examination of the radioactivity in the 0.2 mM calcium nitrate wash solution indicates that most of the ^{14}C-labeled bromacil associated with the surface of the immersed roots was easily washed off but not out of the root apoplast (Table I). The treatment concentration decreased less than 4% over the period of any experiment.

Since changes in root physiology are expected with time and perhaps because of the test chemical, respiration of the roots was examined. Nutrient solution containing a

representative set of plant roots was saturated in a closed system, without air space, and the oxygen depletion pattern was observed. After 24 hr, the solution was re-saturated with oxygen and the test was repeated. Oxygen was depleted from the solution at the rate of 33.5 nmoles g^{-1} hr^{-1} in both the 0–24 hr time period and the 24–48 hr time period. The similar patterns indicate that within this period (48 hr) the roots were still alive and respiring. The effect of bromacil on root respiration was tested in the same manner except rates were observed for only the first 30 min and repeated at intervals. Bromacil at 0.8 μg g^{-1} stimulated respiration as much as 112% but did not yield any apparent damage to the roots, at least within the periods of these studies.

A study of the uptake by 'dead' vs 'live' roots was conducted. 'Dead' roots were prepared by keeping them in oxygen-free nutrient solution (prepared by bubbling with N_2) for 24 hr. Following this procedure, the solution was replenished with O_2. Although there was no evidence of structural damage, the tissues were non-respiring. Living roots accumulated significant quantities of bromacil in both O_2-saturated and O_2-free nutrient solutions (Table II), while the non-living roots did not. This indicates that uptake and retention of bromacil within plant roots depends on living cells.

Uptake of bromacil by barley roots increased with temperature (Table III), yielding a Q_{10} value of 1.5. This also indicates diffusion and argues against active uptake by reactions requiring chemical energy which are characterized by Q_{10} values greater than 2.0.

Many organic compounds are only slightly soluble or are insoluble in water. Representative compounds in the latter category, such as hexachlorobenzene, will be tested

TABLE II

Uptake of bromacil by living or dead (oxygen-starved) barley roots from nitrogen- or oxygen-saturated nutrient solution containing 0.8 mg l^{-1} bromacil. Roots were exposed to bromacil for 30 min

Treatment	Mean bromacil uptake (ng g^{-1} fresh root hr^{-1})	Std. dev.
[a] Dead roots in N_2-saturated solution	79	23
[a] Dead roots in O_2-saturated solution	64	23
Living roots in N_2-saturated solution	357	45
Living roots in O_2-saturated solution	374	56

[a] Roots held in O_2-free solution 24 hr prior to experiment.

TABLE III

Effects of temperature on the uptake of bromacil by isolated barley roots exposed to 0.8 mg bromacil l^{-1} of solution for 2 hr

Temperature (°C)	Uptake of bromacil (ng g^{-1})	Std. dev.
12	247	60
22	334	22
32	543	93

for uptake by excised roots in future experiments. Problems maintaining these chemicals suspended in nutrient solution and in contact with root surfaces must be solved before such substances can be used as test chemicals. These substances can be kept in suspension, and in contact with the roots, by carrier compounds or vigorous agitation.

Other examples of organic uptake experiments show different uptake patterns. These data were useful in planning and conducting this experiment. For example, excised barley roots have been used in experiments with the uptake of the herbicides monuron and 2,4-D (Donaldson *et al.*, 1973). It was established that monuron absorption: (1) is energy-independent; (2) increases only slightly with temperature; (3) increases rapidly at first and then remains constant; (4) does not occur against a concentration gradient; (5) occurs in a linear relationship with external solution concentration; and (6) is not inhibited in the presence of compounds of similar chemical structure (diuron) (Donaldson *et al.*, 1973). On the other hand, 2,4-D absorption: (1) required oxygen; (2) had a high temperature coefficient; (3) had a rate of absorption that was a hyperbolic function of the external concentration; (4) occurred against a concentration gradient; (5) was inhibited by a compound of similar structure (2,4,5-T) (Donaldson *et al.*, 1973); and (6) was inhibited by several compounds known to inhibit enzyme activity (Donaldson *et al.*, 1973). These experiments classify bromacil as similar to monuron in terms of uptake characteristics, i.e., uptake: (1) increases only slightly with temperature; (2) increases rapidly at first and then remains constant; (3) does not occur against a concentration gradient; and (4) occurs in linear relationship with external solution concentration.

The method of studying bromacil uptake used in this experiment has several advantages as a procedure for investigating the uptake of a variety of organic compounds: (1) only 7 days are needed to culture plant material; (2) the culture apparatus occupies a very small space and several plant cultures can be maintained simultaneously; (3) the exposure apparatus can be maintained in a hood and therefore easily conforms to toxic and radioisotope safety requirements; (4) the root material is easily collected after exposure and ashed at the rate of 20–30 samples hr^{-1} in a combustion oxidizer; (5) the radioactivity in CO_2 collected by the absorbing liquid is easily measured; and (6) the test procedure is short and uncomplicated. Many tests can be conducted in a short time. The plants will be relatively small due to the early stage of growth at which they are exposed and only the roots are needed. Therefore, there is no need for expensive and elaborate exposure facilities and equipment to conduct the treatments. There is no need for a greenhouse or growth chambers since test plants can be cultured in a laboratory. While the procedure does not involve whole plants, the information gained by using it will suggest compounds which warrant further testing.

The results of these experiments show that bromacil was passively absorbed by excised barley roots. Our experience indicates that other water-soluble chemicals can be readily investigated by this method.

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